CellPress

Bending Membranes into Different Shapes

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http://dx.doi.org/10.1016/j.str.2015.04.012

BAR domains bend membranes by imposing their curved shape. In this issue, Isas et al. show the structural differences in the interaction of the BAR domain protein amphiphysin with vesicles and tubes. They find that superficial interactions lead to vesicles, whereas more penetrating interactions of a more crowded protein lead to tubes.

The ability to bend and unbend membranes is fundamental to the function of cell membranes. Cell division, endocytosis, and vesicle trafficking are just a few of many cellular processes involving membranes being bent in a controlled manner. Although some membranes spontaneously curve due to inherent properties of their constituting lipids, it is the controlled reshaping of membranes by proteins that is at the heart of cellular functions that require membrane remodeling (McMahon and Gallop, 2005). Many of the shapeshifting proteins that can sense, stabilize, and induce membrane curvature are known. The molecular details of how these functions are achieved at the molecular level, however, remain largely unknown. As with other processes involving intimate interplay between proteins and membranes, the well-established techniques for structure determination yield only limited results. Crystallizing a membrane-bending protein caught in the act of membrane bending is challenging and may even be beyond the ability of current technology. The same can be said about acquiring NMR spectra of sufficient resolution to resolve the protein-membrane interaction at the atomic level. Until the day that electron microscopy provides images of a protein bound to a curved membrane of such exquisite quality as to resolve the molecular details of the interaction, EPR spectroscopy is being successfully employed to these questions and provides some of the best current insight into the molecular basis underlying these important processes (Henne et al., 2007; Jao et al., 2010; Lai et al., 2012; Shah et al., 2014; Varkey et al., 2013).

The BAR (Bin/amphiphysin/Rvs) domain is one of the structural elements known to bend membranes. The structure of the amphiphysin BAR domain provides a beautiful example of how function follows structure (Peter et al., 2004). Two individual domains assemble to form a dimer with a highly suggestive elongated crescent shape and a concave surface containing several patches of basic residues ideally placed to interact with a membrane. The structure thus immediately suggests a membrane-bending mechanism based on scaffolding. The protein is in effect, forcing the membrane to adapt to its own curved shape. This mechanism requires that the internal stability of the dimer and the strength of the protein-lipid interaction can overcome the inherent tendency of the membrane to remain flat. Not resolved in the crystal structure of the amphiphysin BAR domain is an N-terminal sequence that can be shown to undergo a conformational change toward an amphipathic helix upon membrane binding. (The BAR domain together with this helix is referred to as N-BAR.) This structural remodeling of the protein leads to an alternative mechanism of membrane bending—one based on wedging. In this mechanism, the amphipathic helix penetrates the outer leaflet of the bilayer, physically separates lipid headgroups, and occupies the space between them, thereby forcing the membrane to bend. Further structural studies have shown that amphiphysin has a propensity to polymerize at high concentration, thereby creating higher order regular structures that promote tubular membrane shapes.

The work presented by Isas et al. (2015) describes studies that help provide a coherent understanding of the molecular basis of how amphiphysin can bend both vesicular and tubular membranes. Vesicles are isotropically bent, meaning that the curvature at any given position is equal in all directions. In membrane tubes, the curvature changes with orientation and becomes zero along the axis of the tube. The authors spin-label amphiphysin N-BAR at 63 different positions in the terminal helix and across the protein and then study the labeled protein bound to both vesicles and tubes. The authors searched for and found conditions that predominantly form either vesicles or tubes in the presence of protein. In results that are reminiscent of their recent findings on endophilin (Ambroso et al., 2014), the authors find that both the BAR domain and the N-terminal helix interact differently with the membrane in the two substrates. In the absence of membranes, the N-terminal sequence is unfolded, explaining the lack of corresponding density in the electron density map. In the presence of vesicles, the sequence forms an amphipathic helix that is inserted into the headgroup layer of the membrane bilayer. Depth calibration by EPR reveals an immersion of the helix (measured at the center) to about the phosphate layer. Residues on the concave surface of the BAR domain, on the other hand, do not directly interact with the membrane surface; therefore, there appears to be a gap between the two curved surfaces that extends almost to the edge of the protein surface. This lack of interaction between much of the protein surface and the membrane is not consistent with the scaffolding mechanism of membrane bending that flows so easily from the structure of the protein and is more in line with a wedging mechanism. When amphiphysin is bound to tubes, however, the situation changes notably: the whole N-BAR assembly

moves closer to the membrane. The amphipathic helix penetrates even deeper into the membrane (to the level of the acyl chains) and concave surface residues now show clear evidence of direct contact with and, at some sites, even penetration into the membrane. Unlike vesicle binding, these conditions are consistent with a scaffolding mechanism. When the authors reduce the number of proteins per unit surface, they find that conditions that had previously resulted in the formation of tubes now result in a mixture of both tubes and vesicles. These findings are consistent with high protein concentrations biasing the membrane toward a tubular shape.

The emerging view from these and previous studies on endophilin is a set of two distinct modes of interaction between N-BAR proteins and membranes (Figure 1). Under conditions with relatively few proteins per unit surface, the proteins associate superficially with membranes and force vesicular shapes through a wedging mechanism, with individual proteins being oriented in various different directions. When the protein concentration is raised to a level where N-BARs become sufficiently crowded to polymerize, their self-assembly leads to regular ordered arrays in which all long axes of individual domains become vectorially aligned. Individual domains in the assembly move closer to the membrane and impose their shape through a scaffolding mechanism. Because all curved surfaces in the N-BAR superstructure are aligned, the forced shape is that of a tube. Future studies will show whether these two different modes of interaction can be selectively regulated to allow switching between different membrane shapes.

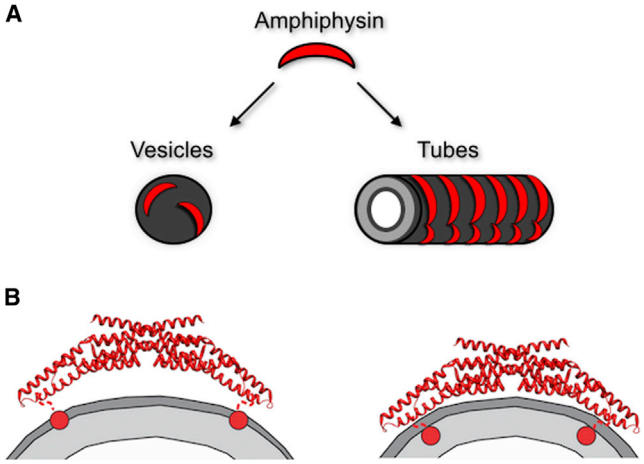

Figure 1. Schematic Illustration of Amphiphysin Binding to Vesicles and Tubes

(A) At low protein to membrane ratios (left arrow), amphiphysin is oriented in different directions and forces the membrane into a vesicular shape. At high protein to membrane ratios (right arrow), amphiphysin polymerizes, and the resulting aligned amphiphysin superstructure forces the membrane into a tubular shape.

(B) On vesicles (left), amphiphysin predominantly uses its amphipathic helices (red circles) rather than the BAR domain for membrane binding. The helices force membrane curvature by wedging into the headgroup region (dark gray). On tubes (right), the BAR domain moves into contact with the lipid headgroups and the amphipathic helices move deeper into the acyl chain region (light gray). For simplicity, only the outer leaflet of the membrane is shown.

Modified from Ambroso et al. (2014) with permission from the authors.

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